AN INVESTIGATION OF SOME FACTORS INFLUENCING LIPASE PRODUCTION BY GEOTRICHUM CANDIDUM

BY

WILLARD OMER NELSON

B.S., University of Illinois, 1937
M.S., University of Illinois, 1940

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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IN THE GRADUATE COLLEGE OF THE
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Introduction

Much of the interest in the lipolytic reaction within the confines of dairy science has been centered upon the nature and activity of the natural milk lipases, and upon fat hydrolysis caused by lipoclastic microorganisms growing in dairy products. Fouts (1940) investigated some of the factors responsible for the variation in acid values of fat in cream and butter, and concluded that *Geotrichum candidum (Oospora lactis), Mycotorula lipolytica,* and other lipolytic microorganisms were of greater significance than milk lipase as a cause of fat hydrolysis in raw cream.

The environment in which microorganisms are grown and the conditions under which the enzyme is tested have been observed to influence the production and activity of microbial lipase. Although the lipolytic properties of *G. candidum* have been known for some time, the literature contains only scant information pertaining to the factors which determine the production and activity of the lipase elaborated by this comon dairy contaminant.

The following study was conducted to ascertain: (1) some of the factors influencing growth and lipase production by *G. candidum,* and (2) some of the factors which influence the activity of this enzyme.

Experimental Methods

The cultures of *G. candidum* used in this study were obtained from the culture collection of the Dairy Science Department, University of Illinois, Urbana, Illinois. These cultures were grown at 20°C. in a hydrolyzed casein basal medium to which had been added the specific nutrient materials under

test. At the end of a 4-day incubation period the amount of growth present was measured gravimetrically by the technic employed by Ellickson (1948). The lipolytic activity of the culture filtrates was estimated by titrating the fatty acids liberated from olive oil substrate by the action of the culture filtrate. The substrate, emulsified in 0.3 per cent agar buffered at pH 6.0, was incubated at 20°C. and aliquots were removed periodically for titration.

Experimental Results

Experimental evidence was obtained to support the hypothesis that the lipase of *G. candidum* is primarily an extracellular enzyme. Extracts prepared from the mycelia exhibited only slight activity in comparison to the lipolytic activity of culture filtrates. Cell autolysis which occurred in aged cultures did not give rise to a large increase in the lipase concentration in the cultures filtrates.

The extracellular lipase hydrolyzed numerous substrates, however natural oils were more readily attacked than were synthetic triglycerides, monoglycerides, and the fatty acid esters of glycol. Maximum lipolysis of butter fat occurred at pH 6.0, although moderate activity was observed at pH 5.0 and at pH 8.0. Lipolysis did not occur in butter fat substrate buffered at pH 4.0. The enzyme was active at 20°C. and 37°C. Maximum fat splitting took when the substrate was incubated at 30°C. Olive oil and corn salad oil were hydrolyzed to a greater extent than was butter oil.

The lipase concentrations in the growth media increased until levels approaching a maximum were encountered on the fourth or fifth day of incubation. Filtrates from cultures which had been aerated during growth were devoid of the lipase. The activity of culture filtrates also was reduced when the filtrate was aerated by agitation. Lipase activity was partially lost when filtrate was saturated with oxygen. Activity was not restored by treating the inhibited lipase solution with glutathione, ascorbic acid, cysteine, and sodium sulfide.

The enzyme was destroyed when culture filtrates were acidified to pH 3.0 with hydrochloric acid and reneutralized to pH 7.0 with sodium hydroxide. The filtrate lipase was not readily amenable to concentration by precipitation with saturated ammonium sulfate.

The various cultures of *G. candidum* examined did not respond alike to the experimental media. The differences noted were primarily quantitative rather than qualitative in nature. All of the cultures were lipolytic and elaborated an extracellular lipase.

The nutritional treatment accorded the cultures of *G. candidum* exerted a pronounced influence on the growth of the organism and the levels of lipase found in the cultures filtrates. Polysaccharides, trisaccharides, and disaccharides were not metabolized and consequently the amount of growth and the lipolytic activity of filtrates obtained from such cultures approximated the results obtained in the basal medium.

Growth was stimulated when galactose, mannose, xylose, levulose, and glucose were added singly to the basal medium. The filtrates from such media exhibited less lipolytic activity than the basal medium filtrate. Glycerol, propylene glycol, and mannitol exerted a similar influence.

The removal of ether soluble materials from the hydrolyzed casein did not alter the growth and lipase producing capacities of *G. candidum* when it was cultivated on a medium composed of the defatted hydrolysate. When the mold was grown on a medium rich in organic nitrogen, the addition of natural fats resulted in growth stimulation and lowered concentrations of filtrate lipase. The data secured by the use of fats in combination with ammonium chloride as the nitrogen source of media suggested that fats may have increased lipase production.

G. candidum was grown in the presence of the sodium salts of various organic acids to determine the influence of these salts on growth and lipase production. Increased levels of growth and decreased concentrations of lipase were encoun-

tered in those media containing lactate and glycerate. A similar response was noted when the potassium salts of the lower molecular weight fatty acids were tested. Growth increased progressively in the presence of the salts of fatty acids of successively longer chain length, up to the seven carbon member of the saturated series. No growth was obtained in the presence of the potassium salts of heptoic, caprylic, nonylic, and capric acids. When three acids of the unsaturated series were tested, growth was increased and lipase decreased in the presence of the crotonic acid salt. The mold did not grow in those media containing undecylenic and oleic acid salts.

The experimental data indicated that the nitrogen component of the medium exerted an influence on growth and lipase production. The mold grew poorly on intact protein materials such as isoelectric casein, sodium caseinate, and gelatin. All of the hydrolyzed protein materials supported moderate to profuse growth, which gave rise to various concentrations of filtrate lipase. Equivalent levels of growth were present in enzyme hydrolyzed casein and acid hydrolyzed casin media. However the filtrate from the enzyme hydrolyzed casein medium contained twice the amount of lipolytic activity that was found in the acid treated material. These results may have been due to the degree of protein degradation which existed in the test materials, or they may have been the result of the destruction of specific compounds during hydrolysis.

In the presence of amino acids, the single addition of selected growth factors had but slight influence on growth and lipase production. The accumulative effects of salts, vitamins, and purine and pyrimidine bases stimulated lipase production.

During the course of the investigation it was demonstrated on numerous occasions that the lipolytic activity of culture filtrates was not directly proportional to the growth of the test organism. In some instances the lipolytic activity of filtrate tended to be inversely proportional to growth. These observations suggested that the constant level of hydrolyzed casein

employed in the basal medium could be the limiting factor. *G. candidum* was grown in three levels of hydrolyzed casein and the results showed that increased concentrations of organic nitrogen yielded increased levels of growth and filtrate lipase. However, lipase production per milligram of mold growth decreased as the hydrolyzed casein component of the medium was increased.

It was established by additional experiments that the nutritional environment and pH at which growth occurred in buffered media did not influence the distribution of lipase between the culture filtrate and the mold mycelia. These experiments did show, however, that there was an optimum pH for lipase production, and that this optimum pH for lipase production varied in accordance with the nutritional treatment of the growing culture. Maximum concentrations of lipase were encountered in the basal medium when it was buffered at pH 5.3 to pH 5.8. Maximum concentrations of the enzyme were found in a glycerol medium when it was buffered at pH 6.7 to pH 6.8. Lipase production in the basal medium occurred over the range from pH 5.3 to pH 8.2. On the other hand, the buffered glycerol cultures produced lipase in a narrower range from pH 6.7 to pH 7.9.

Summary

The lipase produced by *Geotrichum candidum* is an extracellular enzyme which hydrolyzed natural oils more readily than other substrates. Maximum activity occurred at pH 6.0 at 30°C., although moderate activity was observed in substrates buffered from pH 5.0 to pH 8.0 and incubated at 20°C. and 37°C. The enzyme was inactive at pH 4.0 and was destroyed by acidification to pH 3.0 followed by neutralization for assay purposes. The lipase was also sensitive to oxygen, and precipitation with saturated ammonium chloride destroyed a major portion of the activity.

The nutritional treatment accorded the cultures exerted a pronounced influence on growth and levels of lipase found in

the culture filtrates. The carbohydrate component and the nitrogen component of the medium were responsible for significant variations in the data. In the presence of carbohydrates and fats which stimulated growth, lower levels of enzyme were encountered. The optimum pH for lipase production varied with the composition of the growth media. Maximum concentrations of lipase were present in the basal medium buffered at pH 5.3 to 5.8, whereas pH 6.85 was the optimum for lipase production in buffered glycerol medium. The low levels of lipase which occurred in the presence of abundant growth were attributed to: (1) inactivation of the enzyme by the acids formed during carbohydrate utilization, (2) rapid changes in the pH of the growth medium away from the optimum for lipase production, and (3) unknown primary effects contributed by the constituents of the medium.

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VITA

Willard Omer Nelson was born at Urbana, Illinois, on September 27, 1914. He attended elementary and secondary schools in Tuscola, Illinois, where he was graduated in 1932. In September 1933 he entered the University of Illinois, and received a B.S. degree in bacteriology in February, 1937.

The author was employed in the Research and Technical Division of Wilson and Company, Incorporated, Chicago, Illinois, from February 1937 until March, 1943. He received the degree of Master of Science in bacteriology at the University of Illinois, Chicago, Illinois, in October 1940.

In 1943 the author entered military service and served as a laboratory officer in the Sanitary Corps of The Army of The United States. Duty stations included the Army Medical Center, Washington, D.C.; the Second Service Command Medical Laboratory, New York, New York; and the Technical Division, Camp Detrick, Frederick, Maryland.

Upon release from active duty in 1946, the author became a member of the faculty in the Dairy Science department at the University of Illinois. The interim period has been devoted to research, instruction, and graduate study in dairy bacteriology.